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Bedside Measurement of Factor VIII:C Activity in Individuals With Hemophilia A

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Factor VIII replacement therapy for patients with hemophilia A is conventionally monitored using a plasma-based factor VIII:C assay (a modified activated partial thromboplastin time [APTT] test). The plasma factor VIII assay requires the preparation of plasma from citrated whole blood and measurement of the clotting times of mixtures of patient plasma, factor VIII-deficient substrate, and APTT reagent. Results are not routinely available in less than 1.5 hr, reducing the clinical value of the laboratory data regarding the ability to immediately adjust patient therapy. Results from the whole blood factor VIII assay, performed on a portable coagulation analyzer and using test tubes prefilled with the necessary APTT and factor VIII-deficient reagents, are available within 5–7 min. This immediate determination of the factor VIII:C level from citrated whole blood provides the opportunity to greatly reduce turnaround time and improve the efficacy of factor VIII replacement therapy. Based on clotting time, factor VIII:C activity is read from a standard curve. A clinical evaluation of this whole blood test was performed in two hemophilia centers. A high degree of correlation was seen ($r = 0.813$, $n = 220$) between the whole blood values obtained and conventional laboratory results. This level of correlation was superior to that obtained when comparing two different plasma-based systems ($r = 0.753$, $n = 23$). Factor VIII:C activity levels measured using the whole blood assay system were similar, irrespective of the test operator (laboratory technologist, nurse clinician, or patient). This study indicates that the whole blood factor VIII assay provides results comparable to those of conventional plasma-based assays, but in a more rapid and efficient manner. It provides an opportunity to reduce unnecessary patient consumption of replacement preparations, hence reducing the cost of hemophilia A maintenance and prophylaxis regimens, and to reduce overall patient exposure to human blood products.

Key words: factor VIII:C, hemophilia A, bedside assay

INTRODUCTION

The primary objectives in the care of individuals with hemophilia A are the treatment and prevention of hemorrhagic events in a timely manner, thus minimizing the morbidity and mortality associated with this congenital bleeding disorder. The dosing of factor VIII replacement therapy is often calculated empirically, with target values following infusion of 15–30% (0.15–0.3 units/ml) to offset possible mild hemorrhages, or ≥80% (0.8 units/ml) to treat bleeding associated with head injury, surgical trauma, or life-threatening lesions [1]. Furthermore, there are emerging data to indicate that prophylactic replacement regimens which maintain trough factor VIII activity levels ≥1–3% reduce joint damage and the danger of spontaneous life-threatening bleeds into the central nervous system of severely affected individuals [2]. Adequacy of dosing is then critical to assessment of any therapeutic strategy, and must be confirmed via laboratory testing which requires the preparation of plasma from citrated blood and is labor-intensive and time-consuming.

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Due to patient variability of response to infused factor VIII and the delay associated with the receipt of laboratory test values, physicians frequently administer unnecessarily large amounts of factor VIII replacement products in time-critical situations [3]. This practice adds to the expense of overall treatment and often requires the physician to reinfuse the patient if active bleeding is not controlled with the first dose, even though this bleeding may be due to complications not related to factor VIII levels.

The availability of accurate, user-friendly instrumentation to measure factor VIII:C activities at the bedside would be helpful in optimizing both the dosing and cost-effectiveness of hemophilia replacement therapy. It would also improve the level of hemophilia care by aiding the physician in the determination of the cause of bleeding diathesis. This study examines the accuracy, reproducibility and ease of use of a new bedside system which addresses these concerns.

MATERIALS AND METHODS

Patient Population

Individuals with documented hemophilia A arriving for treatment at either George Washington University Medical Center (site 1) or the Hemophilia Center of Western New York (site 2) who were willing to provide informed consent were included in this study. Institutional Review Board approval was received for each hemophilia center. Normal individuals were also included in this study as controls.

Sample Collection

Duplicate blood specimens were obtained via dual syringe specimen venipuncture into blood collection tubes containing sodium citrate (final concentration in blood, 0.38%). One sample was used immediately to perform the bedside factor VIII assay. The duplicate sample was either sent to the on-site laboratory for testing, or centrifuged (2,000*g*, 10 min, room temperature) immediately and the plasma frozen for later testing. In many patients, blood samples were collected both prior to and 20–45 min following infusion of factor VIII replacement products, the dosing of which was dictated by the severity of clinical bleeding episodes.

Factor VIII:C Assay Systems

Conventional factor VIII:C assays were performed on plasma, utilizing three laboratory-based reagent and instrumentation systems. Both the ACL3000+ Automated Coagulation System (International Laboratory, Lexington, MA) and the Labor Coa Screener (Sigma Diagnostics, St. Louis, MO) employ photooptical detection methods, while the ST4 (American Bioproducts Co., Parsippany, NJ) detects clot formation through increases in sample viscosity. In all three systems, the level of factor VIII:C is determined using a modified activated partial thromboplastin time (APTT). Factor VIII:C activity is determined by the ability of the patient plasma to correct the prolonged clotting time of a factor VIII:C-deficient substrate, and by the comparison of this clotting time to a standard curve either generated at time of testing or stored in the coagulation instrument. The APTT reagents employed in this study were the IL Test APTT reagent (ACL3000+, Instrumentation Laboratory, Lexington, MA), Thrombo-Screen Kontact APTT reagent (Coa Screener, Pacific Hemostasis, Ventura, CA), and PTT Automate-5 (ST4, American BioProducts Co., Parsippany, NJ).

Point-of-care determination of factor VIII:C activity was performed using the Whole Blood Factor VIII:C Assay (WBFVIII) and the ITC Whole Blood Coagulation Analyzer (both from International Technidyne Corporation, Edison, NJ). Similar to the plasma-based factor VIII:C assay, the WBFVIII is a modified APTT based on the extent to which a patient's blood corrects the prolonged clotting time of a factor VIII-depleted substrate. The WBFVIII test tube contains a lyophilized preparation of factor VIII-deficient plasma and APTT reagent (platelet factor substitute, blood coagulation activator, and stabilizing buffers). To perform the assay, the WBFVIII test tube is rehydrated with 1.5 ml of calcium chloride solution and preincubated in the test well for 30 sec. One half ml of citrated whole blood is then added to the preincubated tube, and the analyzer timer is started. The clotting time displayed at the completion of the test is used to determine the factor VIII activity level of the whole blood sample from the standard curve of factor VIII activity vs. clotting time supplied with each lot of test tubes. The standard curve is generated by the manufacturer using a whole blood substrate made by combining red blood cells with plasmas of known factor VIII:C activity. WBFVIII assays are performed on these whole blood substrates, which are then centrifuged to obtain plasma samples which are retested for factor VIII:C activity using standard laboratory techniques and a World Health Organization (WHO) standardized reference plasma preparation.

In clinical application, the patient's blood specimen is tested undiluted. If the clotting time of a patient sample corresponds to a factor VIII activity of >40%, the sample is tested again after being diluted 1:1 in a physiologic saline diluent supplied with the test tubes, to obtain a more accurate factor VIII:C activity determination.

Quality control of the assay system is achieved using two levels of control preparation. These factor VIII control plasmas, manufactured for use with the WBFVIII assay, are supplied as assayed, freeze-dried controls containing moderate (0.22–0.40 units/ml factor VIII:C) and low (0.01–0.11 units/ml factorVIII:C) factor VIII:C levels. After rehydration, these plasmas are assayed in WBFVIII tubes, as described for patient blood samples.

An acceptable range of factor VIII:C levels, determined from the standard curve supplied with the test tubes, is supplied with each package of control plasmas.

RESULTS

The precision of the assay was determined using the quality-control plasmas previously described (see Materials and Methods), containing known moderate and low factor VIII:C levels. Multiple determinations over several test days showed high reproducibility (coefficient of variation (c.v.) of 5.3% and 5.1% for normal and abnormal levels, respectively).

Over the course of this study, 220 samples from the two hemophilia centers were analyzed for factor VIII:C activity by both whole blood and plasma assays. The correlation coefficient (r) for all samples was 0.813. Samples containing factor VIII:C levels >40% were assayed in the WBFVIII system after a 1:1 dilution in saline before being included in this analysis. The correlation coefficient value improved at lower levels of factor VIII:C, corresponding to the optimal sensitivity range of the instrument and assay. Figure 1 shows the correlation of the WBFVIII assay with plasma values for patients with factor VIII:C levels <40%. The upper end of this range represents adequate levels for replacement therapy of factor VIII:C after hemorrhagic episodes for hemophilia A patients (Fig. 1A). In this range the overall correlation improves to 0.850. The accuracy of the WBFVIII assay was maintained at the low end of this range, as measured in samples containing <15% (Fig. 1B) or <5% (Fig. 1C) factor VIII:C activity. A clinically significant discrepancy was observed for one sample with <5% factor VIII:C activity. While the laboratory reported a 5% factor VIII:C activity for this sample, the WBFVIII system reported only 1% factor VIII:C.

Differences in the WBFVIII to plasma assay correlation were specific to the plasma analyzer used. One set of patient samples (site 1) was tested using the WBFVIII assay and both the ACL3000+ and the ST4 plasma assay systems. In those patients with factor VIII:C levels <40%, the correlation of the WBFVIII assay to the ACL values (r = 0.830, n = 22) was superior to the correlation of the WBFVIII assay to the ST4 (r = 0.778, n = 23), as well as the correlation of the two plasma systems to each other (r = 0.753, n = 20).

A second set of patient samples (site 2) was examined to determine operator variability in the performance of the WBFVIII assay. Twenty-four patient samples were analyzed by the plasma-based factor VIII:C assay. In conjunction with the laboratory plasma tests, duplicate whole blood samples were run on the WBFVIII system by the laboratory technician performing the plasma assay, or the hemophilia center nurse clinician, or both. As can be seen in Table I, there is no difference in the results among operators.

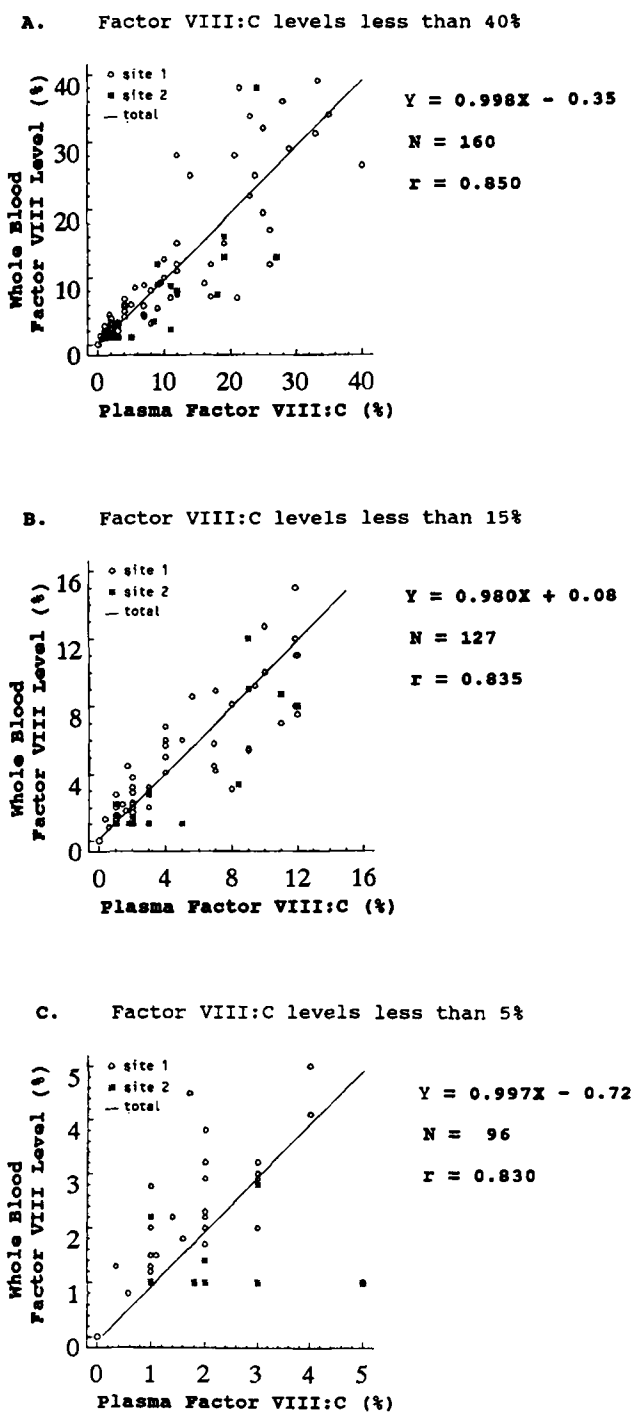

Fig. 1. Correlation of whole blood factor VIII and plasma measurements of factor VIII:C.

Three clinic patients opted to run their own samples on the WBFVIII system both prior to and 45 min after factor VIII infusion. These samples were obtained by the patients themselves via self-venipuncture. As seen in

TABLE I. Influence of Operator on Precision of WBFVIII Assay*

	Technologist (n = 15)[a]		Nurse (n = 23)[a]	
	r	*P*	*r*	*P*
Laboratory[b]	0.900	0.888	0.821	0.347
Technologist			0.888	0.490

*r, correlation coefficient; P, Student's t-test probability.
[a]Test performed at point of care.
[b]Test performed in laboratory.

the technical requirements of the procedure are reduced. Such a system will make patient self-testing a possibility for patients on home care or in a prophylaxis regimen. The excellent sensitivity and precision of the assay at low factor VIII:C levels indicate its usefulness in prophylaxis regimens, with a target level of 3% factor VIII:C [3].

In this study, the sample in which a clinically significant discrepancy existed between the laboratory and whole blood results emphasizes the utility of the WBFVIII assay system. This patient has severe hemophilia A, and at the time of testing had not been infused with factor VIII replacement products for more than 1 week. It is unlikely that this patient's factor VIII:C level was 5%, as reported by the laboratory. The bedside result of 1% factor VIII:C is more consistent with this patient's prior transfusion history.

TABLE II. Accuracy of WBFVIII Assay Performed by Patients*

Patient number	Sampling time	WBFVIII		
		Patient	Nurse	Technologist
1	Preinfusion	11	14	15
	Postinfusion	32	38	NA
2	Preinfusion	24	24	NA
	Postinfusion	49	38	NA
3	Postinfusion	30	35	25

*All values are expressed as percentage of factor VIII:C activity. NA, data not available

Table II, there was no significant difference between the values obtained by these patients and those obtained by the nurse and technologist.

Interinstitutional experience with the WBFVIII system again revealed good correlation with laboratory results. For patients with <40% factor VIII:C activity at George Washington University, a correlation coefficient of 0.91 was obtained from 113 patient samples. The data collected at the Western New York Hemophilia Center displayed a correlation coefficient of 0.892 for 25 samples from patients with factor VIII:C levels <40%.

DISCUSSION

Traditionally, the monitoring of factor VIII:C levels has been accomplished utilizing plasma-based automated coagulation assays which require labor- and time-intensive techniques. In the best of clinical situations, this often requires at least a 1.5–2-hr turnaround time, and impedes the ability of the clinician to determine adequacy of replacement factor dosing in an expedient manner at the bedside or during an emergent situation. In emergency situations a quantitative factor measurement may not be available for several hours or until the next day. The development of whole blood-based test technology provides the means for a rapid measurement of factor VIII:C levels at the patient bedside, in the outpatient clinic, or in an emergency situation. The availability of test results in under 5 min promotes immediate and appropriate dose adjustments. The promising test results obtained by patients has prompted definition of a simpler assay whereby

The availability of reliable and simple methods to measure factor VIII:C concentration allows the health care team to establish the optimal factor VIII:C level for hemostasis, ultimately decreasing the use of clotting factor and improving the cost-benefit ratio for hemophilia care.

This study has demonstrated a very high correlation between the whole blood factor VIII:C assay results and those obtained by conventional automated plasma techniques. It is noteworthy that the correlation of the whole blood and plasma assay results are very similar to the correlation observed for two different plasma assay systems. In a split sample study, the two plasma results were actually more disparate than the whole blood compared to each plasma system independently. The whole blood to plasma correlation improves for factor VIII:C levels in the range of <40% of normal. Patients with factor VIII:C levels at the low end of this range are at greatest risk of experiencing severe hemorrhagic events, and those with factor VIII:C levels at the upper end of this range have attained the desired level for adequate replacement therapy for routine bleeds [1]. Preliminary studies also indicate that the WBFVIII system can accurately measure porcine factor VIII:C levels during infusion (Hyate C, Speywood Pharmaceuticals, Agoura Hills, California) required for treatment of the patient with a factor VIII inhibitor (data not shown). The assay system appears to be extremely user-friendly, with good correlation of results

obtained at two different hemophilia centers by nurses, laboratory technologists, and patients. The availability of quality-control material ensures that an acceptable quality assurance program can be instituted in any alternative testing site. This system should be used in expanded, randomized, controlled studies to evaluate the impact on clinical outcome, particularly in regards to the reduction of factor replacement use, the decrease in cost of maintenance and prophylaxis regimens, the reduction of the incidence of spontaneous bleeding, and the potential to reduce exposure of hemophiliacs to human pathogenic blood-borne nonlipid encapsulated viruses which may be transmitted in plasma-derived replacement products [4–6].

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